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Ocean acidification impacts the embryonic development and hatching success of the Florida stone crab, *Menippe mercenaria*

Philip M. Gravinese*

Department of Biological Sciences, Florida Institute of Technology, 150 West University Blvd, Melbourne, FL 32901, United States

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ABSTRACT

The deterioration of coastal habitats due to anthropogenic activities is being caused by nutrient rich runoff which will likely result in more frequent and severe extremes in seawater pH. The embryonic and larval stages of many coastal species may not have the physiological ability to tolerate these extreme shifts in pH forecasted for future oceans. The stone crab, *Menippe mercenaria*, was used to determine the impact of low seawater pH on embryonic development and hatching success. Ovigerous females were maintained in environments characteristic of both present-day and reduced seawater pH. Lower pH significantly reduced the rate of embryonic development (i.e., time to hatching) by ~24%, but had no effect on the size of developing embryos (i.e., embryonic volume). Larvae that successfully hatched were not morphologically different between treatments, although hatching success was reduced by 28% in lower pH seawater. Hatching success was also more variable in the reduced pH treatment indicating that some broods may be more tolerant to changes in seawater acidity. Variable hatching success under acidified conditions suggests that stone crab embryos may have the capacity to acclimatize to future seawater pH conditions.

1. Introduction

In coastal areas, anthropogenic influences including eutrophication, sewage inputs, and wetland degradation contribute to decreases in seawater pH (Millero et al., 2001; Bauer et al., 2013; Zhang and Fischer, 2014; Ekstrom et al., 2015), and influence the frequency, magnitude, and duration of extreme pH events (Harris et al., 2013; Hauri et al., 2013). The input of land-based runoff into many nearshore ecosystems can reduce seawater pH on daily, monthly, and seasonal temporal scales (Sammut et al., 1995; Zhang and Fischer, 2014). Although nearshore systems, especially estuaries, experience considerable diurnal and tidal fluctuations in pH (Hofmann et al., 2011), future extremes in pH may exceed the critical ecological and physiological thresholds of organisms that have adapted to less extreme environments (Attrill et al., 1999; Ringwood and Keppler, 2002; Hauton et al., 2009). Furthermore, the unprecedented increases in atmospheric carbon dioxide (CO_2) have already decreased seawater pH by 0.1 units (Calderia and Wickett, 2003). Current models forecast additional decreases in seawater pH of 0.14–0.35 units by 2100 and a decrease of 0.77 units within the next 300 years (Meehl et al., 2007; Ekstrom et al., 2015).

Decreases in seawater pH (elevated seawater pCO_2) have been shown to cause a myriad of adverse effects on the behavior, physiology, morphology, and survivorship of calcifying marine organisms (Kurihara et al., 2008; Kroeker et al., 2010; De la Haye et al., 2011). The positive, negative, mixed, and sometimes neutral responses of organisms to lower ocean pH differs among taxonomic groups (Ries et al., 2009; Kroeker et al., 2010) and among populations (Walther et al., 2009). These varied responses make extrapolating the results from elevated seawater pCO_2 experiments challenging when predicting the potential ecological and economic ramifications for a particular species or populations (Miller et al., 2016). Therefore, broad generalizations (i.e., 'winners' or 'losers') about the impacts of elevated pCO_2 on marine biota are problematic as future oceans will likely have some species or populations being more tolerant to lower seawater pH than other species (Carter et al., 2013; Hilmi et al., 2013).

A species' tolerance to low seawater pH may differ through different life-history stages (Whiteley, 2011) and be related to the individual's natural environment (Pane et al., 2008). For instance, low pH conditions impacted the physiology of embryos (e.g., slowed metabolic rate in *Petrolisthes cinctipes*, Carter et al., 2013), and reduced survival in juveniles (*P. cinctipes*, Ceballos-Osuna et al., 2013), but had no noticeable effect in larval survival (e.g., *P. cinctipes*, Ceballos-Osuna et al., 2013). Despite regular exposure to natural changes in pH within the intertidal zone, stage-1 larvae and juveniles of *P. cinctipes* showed enhanced acid-base regulation indicating a greater hypercapnia (e.g., blood pH becomes lower due to elevated CO_2 partial pressures)

* Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, FL 34236, United States.
E-mail address: pgravinese@mote.org.

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tolerance (Carter et al., 2013; Ceballos-Osuna et al., 2013). These results suggest that sensitivity to low pH may vary with developmental stage (Carter et al., 2013; Ceballos-Osuna et al., 2013). Similar mixed effects throughout a species' life-history are also reported in other non-decapod crustaceans (for reviews see Kroeker et al., 2013; Wittmann and Pörtner, 2013). Sensitivity to lower ocean pH conditions may be related to the ability of different life stages to regulate their blood hemolymph pH (i.e., acid-base balance), which can have negative effects on calcified structures leading to morphological abnormalities including reduced body size and spine deformities (Kurihara et al., 2008; Findlay et al., 2009; Long et al., 2013b).

Morphological abnormalities associated with decreases in seawater pH may negatively impact larval survival by reducing body size and defensive spines, which may in turn alter swimming behaviors including the ability to regulate buoyancy, maintain vertical position, and avoid predators. The varied responses to lower ocean pH reported for decapod crustaceans suggests that species-specific experimentation across different life history stages is still necessary to predict how species will respond to more frequent and extreme changes in seawater pH, especially in regions where anthropogenic activities continue to degrade coastal habitats (Bauer et al., 2013; Ekstrom et al., 2015; Miller et al., 2016). Identifying an organism's tolerance to extremes in seawater pH throughout different stages of its life history allows for determining if the species will be able to tolerate and eventually adapt to future decreases in seawater pH (Kurihara et al., 2008).

The Florida stone crab, *Menippe mercenaria*, is an important fishery in the southeastern United States. In Florida alone, stone crab landings support on average a US ~$25-million-a-year commercial and an active recreational fishery (FWC, 2000–2016). The stone crabs embryonic development, larval release, and post-larval recruitment occur within nearshore regions (Lindberg and Marshall, 1984; Krimsky and Epifanio, 2008; Krimsky et al., 2009; Gandy et al., 2010) some of which are being threatened by anthropogenic activities. For example, since 2005 the western fringes of Florida Bay (Florida, USA), have experienced a rate of decline in pH that is estimated to be three-times faster than the rate observed in the open ocean (Zhang and Fischer, 2014). Given the potential mixed effects reduced seawater pH may have on the development of decapod crustaceans, lower ocean pH could have damaging ramifications for economically important species like the Florida stone crab. This study examined the effects of low seawater pH on the embryonic development and hatching success of *M. mercenaria*. The hypothesis that exposure to acidified seawater conditions during embryonic development results in morphological abnormalities in newly-hatched larvae was also tested.

2. Materials and methods

2.1. Study site, collection and maintenance of experimental animals

All experiments were conducted from June–August 2012 at the Mote Tropical Research Laboratory in Summerland Key, Florida, USA (24° 39.69′ N, 81° 27.25′ W). Ovigerous *M. mercenaria* (carapace width $\bar{\mu}$ = 9.56 cm ± 1.06 SD) were collected within 17 km of the coast using commercial stone crab traps at different times throughout the summer. Following transport to the laboratory, crabs were randomly assigned to either the control (present-day pH) or low pH treatment. A total of 16 ovigerous stone crabs were caught in state approved commercial traps; eight were randomly assigned to the control treatment and eight were randomly assigned to the experimental treatment. Each brood was considered one replicate, thus N = 8 in each treatment level. The same eight females in the control and experimental treatment were used to evaluate responses to low seawater pH for the experiments listed below, and were run in parallel (i.e., balanced throughout experimentation) to ensure that differences in brood quality across the season did not bias the results (Supplemental Table 1). Only females with egg masses containing early-stage embryos (i.e., orange egg masses; Supplemental Table 2) were used in experiments. Ovigerous crabs were maintained in individual (40.6 × 20.3 × 25.4 cm) flow-through aquaria that hold 19 L of seawater (salinity 35) under a 14 h to 10 h light:dark cycle that approximated the photoperiod at the time of collection. Water temperatures were maintained within a narrow range (median ± median absolute deviation, MAD) by partially submerging the aquaria in a thermostatically controlled water bath, and approximated the median temperature at the collection site. Crabs were fed frozen shrimp every other day ad libitum, and aquaria were cleaned daily to remove wastes and uneaten food.

2.2. Experimental setup and seawater chemistry

Experiments were performed using a flow-through ocean-acidification system at the Mote Tropical Research Laboratory in Summerland Key, Florida. The system consisted of experimental aquaria (40.6 × 20.3 × 25.4 cm) that were sealed with acrylic lids. Treatment conditions within each aquarium were maintained by pumping seawater through 0.6 cm diameter inlet and outlet holes that were drilled into the acrylic lids. Saltwater for the control (pH = 8.0, ~540 μatm) and low pH treatment (pH = 7.5, ~2100 μatm) were obtained from a saltwater aquifer. The saltwater contained naturally high levels of CO_2, which resulted in a source of saltwater with a pH of 7.5 (± 0.07 SD), and a salinity of ~36 (± 0.6 SD) (Hall et al., 2012). Before being pumped into the system, saltwater passed through sand filters (100 lb) and biological filtration (3.8 cm Bio Balls) to remove particulates and nutrients. Next, saltwater was pumped into a second set of holding reservoirs (1000 L) where CO_2 was degassed with ambient air until near-present day (control) or future pH levels were achieved. A pH/CO_2 controller (Milwaukee, Aquatic Ecosystems, ± 0.2 accuracy, Apopka, FL) was used to regulate the CO_2 levels in each treatment.

Saltwater pH within each experimental chamber was monitored daily using a pH probe (SevenGo pro, Mettler-Toledo, ± 0.002 pH, Germany). Prior to use, a daily three-point calibration (at 26 °C) was performed on the pH probe using NBS buffers. Daily flow rates (ml min^{-1}) were measured by sampling the volume of seawater flowing into each experimental aquarium at ten-second intervals. Salinity of each aquaria for both treatments was monitored daily using a refractometer (resolution 0.2%, LW Scientific, Lawrenceville, GA), and temperature of each aquaria for both treatments was monitored using the same pH probe previously described. Water samples for total alkalinity (A_T) and phosphate measurements were collected from each experimental aquaria at the start (day 0), middle (~day 7), and at the end (after hatching, days 10–12) of each experiment. These water samples were stored at 4.4 °C for A_T and < − 40 °C for phosphate. All water samples were analyzed for total alkalinity within 10–14 days of sampling according to Environmental Protection Agency (EPA) approved method SM 2320 B-1997. Alkalinity samples were stored at 4.4 °C to reduce chances that A_T levels change due to contamination or microbial activity as HgCl was not available.

All seawater samples were processed by the Mote Marine Laboratory Analytical Chemical Ecology facility in Sarasota, FL. Phosphate levels were analyzed using a nutrient auto-analyzer (Bran Luebbe) according to Environmental Protection Agency (EPA) methods 353.2. A gran-titration method (EPA approved method SM 2320 B-1997) was used to estimate A_T values. Titrations were calibrated using a primary grade 0.05 N Na_2CO_3 solution. Three separate aliquots of the Na_2CO_3 solution were titrated to compute the normality of the acid. The average acid normality and ml of titrant were then used to compute A_T. Total alkalinity and pH values (NBS scale) were used to estimate pCO_2 levels for the control and low pH treatment using the CO2Sys program (v2.1; Pierrot et al., 2006). Stoichiometric dissociation constants defined by Mehrbach et al. (1973) and refit by Dickson and Millero (1987) were used during the CO2Sys calculation. Phosphate levels were incorporated into the CO2Sys calculations to better characterize the carbonate system during these experiments.

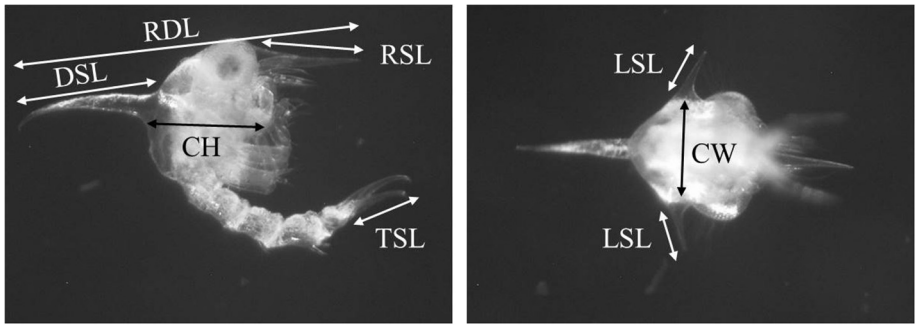

Fig. 1. Measurements made on stage-1 larvae for morphometric analysis included the carapace width (CW), carapace height (CH), lateral spine length (LSL), rostral spine length (RSL), rostro-dorsal length (RDL), dorsal spine length (DSL), and terminal spine length (TSL).

2.3. Embryonic development and hatching success

To monitor and compare the rate of embryonic development of stone crab embryos between the two treatments, we used a 7-stage classification system based on visual inspection of yolk content, eyespot size and shape, presence of abdominal segments, and presence of pigment cells (Supplemental Table 2; Pandian, 1970; Helluy and Beltz, 1991; Garcia-Guerrero and Hendrickx, 2004). The embryonic stage of development of each female used in the control (n = 8) and in the low pH treatment (n = 8) were estimated by removing a subset of 20 embryos from the egg mass of each brood daily. Embryos were removed according to methods outlined by Saigusa (1992) in which embryos were cut to include the ovigerous hairs to minimize damage and disruption to the remaining embryos. Stone crab females are estimated to produce 350,000 eggs in one brood (Noe, 1967). Removal of embryos for all experiments in this study used well under 1% of the total egg mass. Embryos were then photographed at 25 × magnification with a digital camera (Nikon Coolpix 995, Melville NY) attached to a dissecting microscope (Meiji Techno, San Jose CA), and their development stage was recorded (sensu Supplemental Table 2). A generalized linear mixed effects model was used to determine if the rate of development differed among treatments with pH as the fixed factor and brood treated as a random factor. An F-test was then used to determine if the treatments had a significant effect on the embryonic development.

The effects of low pH on embryo size, throughout development, was compared by measuring the diameter (d) near the center axis of each egg (20 eggs per day per female in each experiment) using digital analysis software (Image J). Embryo volume (V) was then estimated using the formula for a sphere:

$$V = \frac{4}{3}\pi\left(\frac{d}{2}\right)^3. \quad (1)$$

A generalized linear mixed effects model was also used to compare the embryo volume with pH as the fixed factor and brood treated as a random factor.

Hatching success was determined by removing a sample of eggs (~100) from the egg mass of the same females used in the low pH treatment (n = 8) and the control (n = 8; according to methods described by Saigusa, 1992). Eggs were removed < 24 h prior to the anticipated time of larval release (Krimsky et al., 2009). Embryos that were < 24 h from hatching were determined from visual examination of the sponge (dark brown-green color) and by microscopic inspection of the yolk content. The timing (in days) of these benchmarks (e.g., development of the eyespot) was consistent in their appearance pre-hatching. Clusters of removed embryos were maintained in clear acrylic chambers (3 cm × 3 cm × 4 cm), and the chambers paralleled the ovigerous female's tank to receive the same flow conditions as the brooding female. Hatching success was determined by counting the total number of hatched larvae 1 h after the first larvae hatched from the isolated egg cluster. Hatching success did not meet the assumption of homogeneity of variance and so the data were ranked (Conover and Iman, 1981). Differences among treatments were determined using a generalized linear mixed effects model with pH as a fixed factor and brood treated as a random factor.

2.4. Morphological analysis of larvae

To determine the potential effects of low pH on larval morphology, twenty newly hatched larvae from each brood used in the control (n = 8) and low pH treatment (n = 8) were collected within 24 h of hatching and were preserved in 5% buffered formalin following the protocols of Webb et al. (2006). Each larva was photographed at 25 × magnification using a digital camera (Nikon CoolPix 995, Melville NY) attached to a dissecting microscope (Zeiss 2000-C, Edmund Optics, Barrington, NJ). Seven morphological features, including the carapace width (CW), carapace height (CH), lateral spine length (LSL), rostral spine length (RSL), rostro-dorsal length (RDL), dorsal spine length (DSL), and terminal spine length (TSL), were measured from the digital micrographs using Image J software (Fig. 1). Because of the high degree of shared variability among morphological features, Principle Component Analysis (PCA) was used to establish a new set of orthogonal variables that were compared among treatment groups. A scree plot was used to determine the number of principle components (PCs) to retain. The derived component scores were then analyzed using separate nested ANOVAs (again, with brood as a nested factor) to determine if larval morphology differed between treatments (Swiney et al., 2015). Because six statistical tests were performed on the same population of ovigerous females used during the aforementioned experiments the critical P-value for this study was adjusted to $P < 0.008$ to minimize type I error.

3. Results

3.1. Water chemistry and experimental conditions

Seawater flow rates through the control and low pH aquaria were 12.7 L h^{-1} ± 2.0 (μ ± SD) and 12.7 L h^{-1} ± 2.3 respectively. The pH was lower in the experimental treatment (7.5 ± 0.05; median ± MAD) compared with the control (8.0 ± 0.06), whereas the total alkalinity (A_T) remained similar between treatments (Supplemental Table 3). Because the measured seawater chemistry variables all have different units and ranges, the coefficient of variation (CV) was used for standardizing the discussion of their relative variability. The CV showed that temperature, salinity, A_T and pH were maintained within narrow ranges in both the low pH (CV in elevated pCO_2: temperature = 1.04%, salinity = 0.40%, TA = 1.12%, pH = 0.27%) and the control (CV in control: temperature = 1.01%, salinity = 0.49%, TA = 1.17%, pH = 0.62%; Supplemental Table 3). The medians (± MAD) for the water chemistry parameters are included in Supplemental Table 3.

The relatively large range of pCO_2 values reported here (Supplemental Table 3) were likely the result of using the NBS pH standards for probe calibration (NBS scale can vary ± 0.05 pH units). Despite the range of pCO_2 values observed during this study, the control

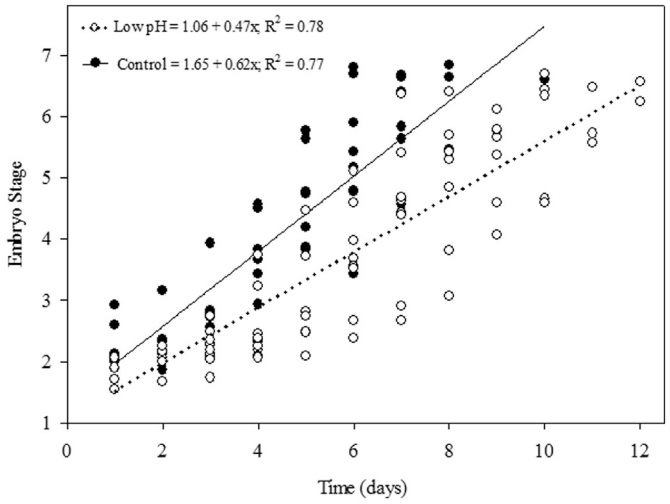

Fig. 2. Change in embryo stage as a function of time for crabs subjected to low pH and control conditions. A linear function was used to determine the rate of change in development among both treatments.

was comparable to pCO_2 ranges reported for some stone crab habitats along the fringes of Florida Bay (Millero et al., 2001; 325–72 µatm). A_T values in the control were also within ranges reported for stone crab habitats along the fringes of Florida Bay, which range from 2000 to 5600 µmol kg^{-1} for A_T (Millero et al., 2001; Dufroe, 2012). Phosphate levels were elevated compared to the range of values reported in Millero et al., 2001 (low pH phosphate = 0.50 µmol kg^{-1} (± 0.08); control phosphate = 0.50 µmol kg^{-1} (± 0.07); Millero et al., 2001 range for ammonia 0–0.24 µmol kg^{-1}). The phosphate values reported here were comparable among the experimental treatment and the control and were not statistically different (*t*-test; P = 0.76) so it is unlikely that the slightly elevated nutrient levels impacted the results of this study.

3.2. Embryonic development and hatching success

Embryos exposed to the low pH treatment developed significantly slower (~24%; calculated using the slopes of the regression lines) than the control conditions on average ($F_{2,\ 136}$ = 20.9, P < 0.001; Fig. 2). Embryonic development took on average 7.3 days ± 1.3 (SD) to complete in the control, and 10 days ± 1.8 to complete in low pH.

The slower development in the low pH treatment resulted in embryos hatching ~2.5–3.5 days later than embryos in the control. The size of embryos and the rate at which the embryo volume increased, at each developmental stage, was not statistically different among treatments ($F_{2,\ 92}$ = 0.53, P = 0.59; Fig. 3). Hatching success varied among broods and ranged from 18%–85% in the low pH treatment compared with 66%–87% in the control. Median (± MAD) hatching success in the low pH treatment was 46.3% (± 16.05) and was significantly reduced compared with the control 74.7% (± 5.1; $F_{1,\ 15}$ = 10.3, P = 0.006; Fig. 4). The decrease among treatments represented a 28% reduction in hatching success.

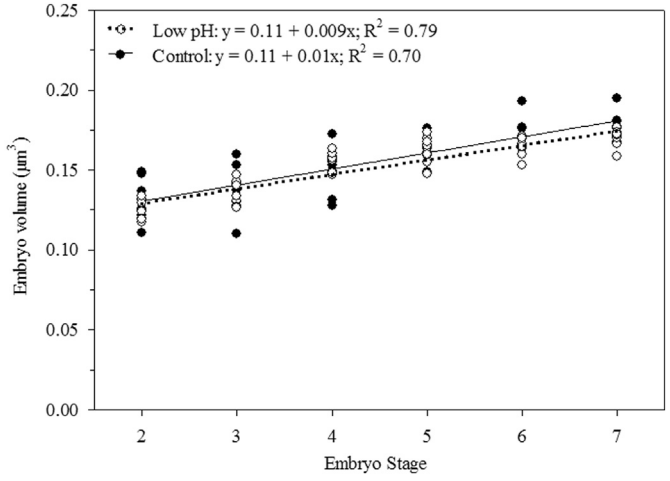

Fig. 3. Change in egg volume at each embryo stage for crabs subjected to low pH and control conditions. A linear function was used to determine the rate of change in embryo volume among both treatments.

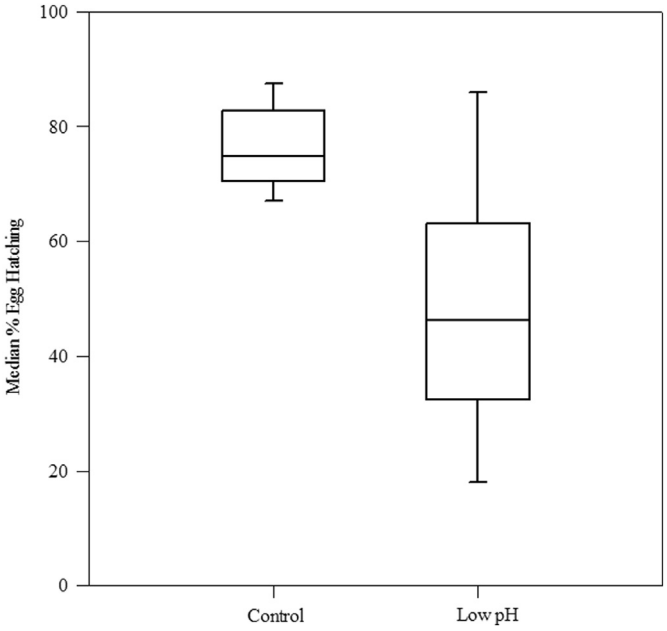

Fig. 4. A box and whiskers plot showing the hatching success of crabs maintained under control and low pH treatment. Horizontal lines within the box indicate the median.

3.3. Larval morphology

PCA analysis on the seven morphological measurements of newly hatched larvae resulted in three principle components (PCs) representing 73% of the variation (Table 1). PC 1 loadings were

Table 1
Results of a principal component analysis on seven morphological characters of newly hatched *M. mercenaria* larvae. Three orthogonal principal components (PCs) representing 72.9% of the total variation were retained by the analysis. The derived component scores were analyzed using separate nested ANOVAs to test for the effects of elevated pCO_2 on larval morphology. Factor loadings > 0.75 were used to determine which variables contributed to each PC.

PC	Eigenvalues	% variation	% cumulative variation
1	2.04	26.2	26.2
2	1.83	29.7	55.9
3	1.21	17.0	72.9

Variable	Loadings		
	PC1	PC2	PC3
Carapace width	0.36	0.41	0.17
Lateral spine (right)	0.95	0.01	0.05
Lateral spine (left)	0.83	0.09	0.48
Rostral spine	0.12	0.98	0.01
Dorsal spine	0.22	0.24	0.05
Rostro-dorsal spine	− 0.05	0.92	0.09
Carapace height	0.20	0.05	0.97

associated with the lateral spine length was interpreted as representing overall larval width. PC 2 loadings were associated with rostro-dorsal spine length, rostral spine length and dorsal spine length, and were interpreted as representing overall animal size, whereas the loadings for PC 3 were interpreted as being associated with carapace height. The derived component scores showed no significant differences among treatments for larval morphology (PC 1: $F_{1,15} = 1.4$, $P = 0.25$; PC 2: $F_{1,15} = 1.1$, $P = 0.32$; PC 3: $F_{1,15} = 0.33$, $P = 0.67$). There was also no significant brood effect on larvae (PC1: $F_{1,15} = 3.2$, $P = 0.07$; PC2: $F_{1,15} = 1.2$, $P = 0.29$; PC3: $F_{1,15} = 0.08$, $P = 0.79$).

4. Discussion

The Florida stone crab *M. mercenaria* was used to assess the potential effects of low pH projected for future oceans on embryonic development, hatching success, and larval morphology in decapod crustaceans. It was anticipated that stone crab embryos would be tolerant of low pH because brooding occurs in nearshore areas where seawater carbonate chemistry and pH vary naturally. Although the sample size was small, acidic seawater impacted both the embryonic development rate and larval hatching success.

The development rate of stone crab embryos was significantly slower (24%), or about 3 days longer, under lower pH. The only other study to show that acidic conditions had a direct effect on the time to hatching in a commercially important decapod crustacean was on the Dungeness crab, *Cancer magister*, and it reports a similar delay in the embryonic development rate (delay by 24%; Miller et al., 2016). Studies on other crustaceans demonstrated that low pH results in a similar delay on embryonic development in the amphipod *Echinogammarus marinus* (~8.5%; Egilsdottir et al., 2009), and the barnacle *Semibalanus balanoides* (~19%; Findlay et al., 2009). Ocean acidification (OA) induced hypercapnia has been shown to reduce metabolism throughout embryogenesis (Ceballos-Osuna et al., 2013). The hypercapnic conditions associated with acidified conditions may impair the ability of embryos to exchange ions or gases (O_2 and CO_2) with the surrounding environment, resulting in extracellular acidosis and lower oxygen consumption rates. For instance, Carter et al. (2013) found that embryo oxygen consumption of the porcelain crab, *Petrolisthes cinctipes*, was 11% lower under low pH conditions. Therefore, exposure of stone crab embryos to lower pH may reduce metabolic rates of oxygen consumption, which would contribute to the slower developmental rates reported here.

Hatching success of stone crab larvae declined by 28% in lower seawater pH (Fig. 4). This reduction is less than what has been reported for some other crustaceans under similar pH conditions, including the barnacle *S. balanoides* (60%; Findlay et al., 2009), the copepod *Calanus finmarchicus* (86%; Mayor et al., 2007), and the Tanner crab, *Chionoecetes bairdi* (40%; Swiney et al., 2015). However, the hatching success results reported here differ from what has been reported for other crab species (*P. cinctipes*, and *C. magister*), which did not show a decrease in the percentage of larvae that successfully hatched under low pH exposure (Ceballos-Osuna et al., 2013; Long et al., 2013a; Miller et al., 2016). Although the underlying cause for the lower hatching success is unknown, other researchers have suggested that embryos could be undergoing extracellular acidosis or CO_2 toxicity (Ceballos-Osuna et al., 2013). During normal development, the oxygen consumption of crustacean embryos increases toward hatching (Brante et al., 2003; Naylor et al., 1999). Therefore, if embryos closer to hatching require more oxygen, and are unable to effectively exchange respiratory gases, then higher H^+ concentrations and/or CO_2 toxicity associated with low seawater pH may limit successful hatching. In contrast, more variable hatching success and a lack of increase in egg volume was observed under low pH conditions in *P. cinctipes*, however, Ceballos-Osuna et al. (2013) did not observe a difference in hatching success among treatments. In this study, the embryos appeared to develop normally (i.e., formation of the limbs and the eye spot), albeit slower in the low pH treatment. Moreover, the embryo size was similar between treatments suggesting that the reduction in hatching success was not linked to the embryonic development.

Interestingly more variable hatching success was observed in the low pH treatment which is likely the result of brood specific responses to pH and indicates that some individuals (broods) were more tolerant to future increases in seawater acidity than others. Variable responses to pH among broods could be related to variation in the carbonate chemistry within habitats where females were collected, especially since seawater carbonate variables within some stone crab habitats have been documented to vary both spatially and temporally (Ceballos-Osuna et al., 2013; Millero et al., 2001; Dufroe, 2012). Although brood quality decreases throughout the spawning season, it is not likely that the collection of ovigerous females during different times throughout the summer contributed to the observed variability in hatching success as late summer broods in the control still exhibited a high percentage of hatching (see Supplemental Table 1). Rather, the variability in hatching success supports the hypothesis that the environmental conditions experienced by the brooding female may indeed influence the embryo's tolerance to environmental fluctuations throughout the brooding period (Sibert et al., 2004; McCormick and Gagliano, 2008; Parker et al., 2012). For instance, the tolerance of some broods to OA conditions could be the result of some female stone crabs experiencing low seawater pH during oogenesis; however, this has not been tested in crustaceans (Carter et al., 2013). The physiological and genetic characteristics of the breeding female (parental genetic input, energy allocated to eggs, exposure to prior environmental stress) can play a role in the viability and quality of the offspring (Sibert et al., 2004; McCormick and Gagliano, 2008). Recent work has indicated that variability in an organism's response could represent the potential for acclimatization among some groups (Sunday et al., 2011; Parker et al., 2012).

Another potential factor contributing to the slower embryonic development and lower hatching success could be the impact of low pH on egg mass care and maintenance by the gravid female. Environmental stressors (like pH) are known to negatively impact normal physiological function and reproduction in some adult crustacean species, including the lobster *Nephrops norvegicus* (Hernroth et al., 2012), the shrimp *Palaemon pacificus* (Kurihara et al., 2008), and the crabs *Chionoecetes tanneri* (Pane et al., 2008), *Necora puber* (Spicer et al., 2007), and *P. cinctipes* (Paganini et al., 2014). Abiotic factors experienced during brooding, such as extremes in salinity, temperature, and pH are also known to slow hatching and reduce the rate of embryonic development (Petersen, 1995; Giménez, 2002). Brood care in crustaceans already comes at a high cost to the female, resulting in a decrease in energetic reserves (i.e., carbohydrates, lipids, and proteins; Fernandez and Brante, 2003; Guadagnoli et al., 2005). Female stone crabs may have difficulty in regulating their internal acid-base balance (like *Necora puber*; Spicer et al., 2007), which would most likely come with an increase in energetic cost, with less energy being allocated to other physiological functions (Whiteley, 2011; Miller et al., 2016). For instance, active brood maintenance by the female crabs during embryonic development facilitates gas exchange and hatching (Wheatly, 1981; Fernandez et al., 2000). Previous work has shown that unventilated eggs during brooding (Naylor et al., 1999), and a lack of brood maintenance by the female (Förster and Baeza, 2001), results in an extended development period, reduced oxygen uptake by the embryos, and a lower hatching rate. Therefore, if acidified seawater results in a reduction in the female's physiological condition (or activity), then lower seawater pH could have a negative impact on brood maintenance, including a decrease in abdominal pumping and egg mass preening, which could reduce the development and hatching success. However, this hypothesis has not been tested experimentally.

The morphology of newly hatched stone crab larvae was not impacted by lower pH. This result is similar to other studies on early life-history stages of larval crustaceans which have shown that the carapace length of newly hatched larvae in the European lobster, *Homarus*

gammarus, and in *C. magister* were similar regardless of seawater acidity (Arnold et al., 2009; Miller et al., 2016). Walther et al. (2010) also showed that after exposure to low pH (elevated pCO_2) conditions newly hatched *Hyas araneus* larvae exhibited no morphological change after hatching, a trend which continued throughout larval development. The results of the morphology experiment are in contrast with Long et al. (2013a), who showed that *Paralithodes camtschaticus* larvae increased their carapace size by 4% when exposed to low seawater pH. This neutral result may stem from the fact that planktonic decapod larvae have unmineralized exoskeletons, thereby reducing the potential adverse effects of low pH on calcification and growth throughout ontogeny (Anger, 2001; Whiteley, 2011). However, there is potential for lower seawater pH to impact the shape, size, or thickness of later stage larvae as more calcium is incorporated into the exoskeleton (Richards, 1958; Neufeld and Cameron, 1992; Arnold et al., 2009; Whiteley, 2011). For example, CO_2-induced acidification caused a reduction in the concentration and in the percentage of calcium found in the exoskeleton in late stage (stage IV) *H. gammarus* larvae (Arnold et al., 2009).

5. Conclusion

This is the first study to determine how increased seawater acidity may impact the embryonic development and hatching success of commercially important stone crabs. A significant reduction in hatching success and slower embryonic development was observed in ovigerous females exposed to low pH conditions for the entire brooding period; however the magnitude of the response was brood specific. This variability among females (e.g., female investment in eggs, potential genetic variation among broods) suggests that some individuals may be more successful than others in their ability to tolerate changes in seawater acidity, and that the species may have the potential for acclimatization (Sunday et al., 2011; Parker et al., 2012). These results indicate that future changes in seawater pCO_2 may reduce the reproductive success of stone crabs, which could have a damaging effect on the sustainability of the stone-crab fishery by potentially limiting larval supply, and creating a bottleneck for the stone crab fishery during early-life stages, especially if pCO_2 levels in nearshore habitats continue to increase. Furthermore, population level effects could be additive if stone crab larvae experience increases in mortality under lower ocean pH or exposure to multiple stressors (i.e., OA and temperature).

Although morphology remained unaffected in newly hatched larvae, it is unclear if larval mortality or physiology will be affected during later larval development. Experiencing such conditions during embryonic and larval development may result in carry-over effects and indirectly affect performance in later life stages (juveniles or adults; Giménez, 2002; Giménez and Anger, 2001). If these negative impacts are exacerbated by carry-over effects, such as high post-hatch mortality, or impaired physiology throughout juvenile development, then recruitment of new individuals into the fishery may also become limited (Schiffer et al., 2013).

Abiotic environmental conditions experienced during embryonic development can alter metabolic processes and individual growth rates which may come with physiological costs such as depleting energy stores that may be required in later developmental stages (see Byrne, 2011 for review, Schiffer et al., 2013). For instance, studies have demonstrated that some species (*H. araneus*) can exhibit high juvenile mortality under low seawater pH despite being exposed to similar conditions in earlier developmental stages (Walther et al., 2010, 2011; Schiffer et al., 2013). Therefore, future work should consider determining if there are any carry-over effects among life stages and how different life history stages may differ in their sensitivity to changes in seawater acidity to ensure the persistence of this valuable species under future acidification.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jembe.2017.09.001.

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